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Chromatographic steroid hormone analyses in treatment of breast cancer and some endocrine diseases

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Document Version

Publisher's PDF, also known as Version of record

Publication date:
1994

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Dikkeschei, L. D. (1994). Chromatographic steroid hormone analyses in treatment of breast cancer and some endocrine diseases. Groningen: s.n.

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For women living in industrialized countries breast cancer and cardiovascular disease are the two most prominent causes of death. In the Netherlands approximately 7.500 new cases of breast cancer are diagnosed every year and nearly 3.500 patients die due to metastatic disease.

Malignant tumor growth is the result of unbridled cell division. Non surgical treatment strategies, like radiation and chemotherapy interfere directly in the cell cycle. Since these interferences are not confined only to malignant cells, growth of normal cells is also inhibited. To minimize side effects and to optimize growth inhibition of malignant cells, more specific interventions are needed.

Chapter 2 summarizes the present knowledge about hormonal therapy in breast cancer treatment. For many decades breast cancer growth has been shown to be estrogen dependent. The principal aim of hormonal therapy is to prevent estrogen mediated tumor growth by inhibiting estrogen synthesis or estrogen binding to the cellular receptor. Nowadays in postmenopausal patients with advanced breast cancer specific estrogen receptor blocking agents is the first line of treatment. Inhibition of estrogen synthesis has gained much attention in recent years and the specificity of this therapy has improved substantially. The aim of the studies described in this thesis was to develop and improve analytical procedures able to measure changes in steroid hormone concentrations during various hormonal therapies.

Chapter 3 describes a method to quantify medroxyprogesterone acetate (MPA) in serum of patients treated with this synthetic steroid. After addition of the internal standard (medroxyprogesterone propionate), MPA was extracted from serum samples, derivatized with trifluoroacetic acid and analyzed by gas chromatography mass-spectrometry (GC-MS). Subsequently the assay was used to tackle the problem whether differences in therapy response could be due to differences in resorption and/or metabolism of the orally administered MPA. Although a dose concentration relation was found, large variations in the maximal serum concentrations reached after one standard dose occurred, explaining a great deal of the wide concentration range found among patients treated with the same daily oral MPA dose. The described assay could therefore be useful in individual adjustment of oral dosages MPA. Levels found by a radioimmunoassay correlated well with the GC-MS assay.

Chapter 4 describes the analysis of megestrol acetate (MA) and cyproterone acetate (CPA), two other synthetic steroids clinically tested in the treatment of breast cancer. In the analysis of MA, CPA is added to the serum sample as internal standard and vice versa. After extraction, concentrations were determined by high performance liquid chromatography (HPLC) with ultraviolet detection. The assay was validated by GC-MS. As for MPA, large differences in concentrations were found in patient receiving the same oral dose of either MA or CPA. The dose concentration relation seen in both cases seems to be nearly linear.

Chapter 5 contemplates the relation between serum concentrations and the bioavailability of MPA and MA. The latter was assessed by determining the effect of each drug on serum concentrations of the adrenal steroids cortisol (C), dehydroepiandrosterone sulfate (DHEAS) and androstenedione (A). Maximal serum concentrations after ingestion of respectively 80 and 500 mg of MA and MPA were found within three to four hours and resulted in a 50% suppression of A and C. A twofold increase in these doses did not result in a further reduction of A and C. Administration of 160 mg MA was as effective as 1000 mg MPA, although peak serum concentration levels were approximately tenfold higher for MA.

Chapter 6 demonstrates the clinical use of the CPA assay as described in chapter 4, in a phase II study. In comparison with MA and MPA, the resorption of CPA was more effective leading to higher serum concentrations during treatment. The concentration range was wide and a twofold rise in serum concentration was found after doubling the oral dose of CPA. Corticoid effects were not observed since A and C were not suppressed. DHEAS was found to be suppressed in a dose dependent manner. In addition LH and FSH levels were reduced whereas prolactin levels were elevated. The therapeutic effect of CPA treatment was rather disappointing, side effects were frequent and sometimes severe. Therefore it was concluded that the drug should not be considered as an alternative for MA and MPA in the hormonal treatment of metastatic breast cancer.

Chapter 7 describes a candidate reference method for the analyses of cortisol, progesterone, estradiol and testosterone. In this rather complex analytical procedure steroids were extracted from serum samples, separated on HPLC, derivatized and quantified by means of GC-MS. This method was used to provide external quality control samples with GC-MS target values.

immunoassays, were compared with the GC-MS target values found. In general, relatively large differences in accuracy and precision were observed and overall means, which were often determined by one or two market leaders, did seldomly correspond satisfactorily with the GC-MS target values. For estradiol, the most relevant assay in the context of this thesis, performance of the immunoassays differed markedly. In addition, these assays were not developed to analyze estrogen levels in the low postmenopausal range. In the evaluation of hormonal therapies it is often necessary to determine a small difference in already low concentrations of estrogens and estrogen conjugates. Therefore, commercially available immunoassays should be carefully selected and thoroughly tested for this purpose. In many cases precise "home made" research immunoassays have to be used. Since GC-MS has the potential to add accuracy to results obtained with these methods we have further developed the assay described in this chapter (see chapter 10).

Chapter 8 describes a procedure for the analysis of Δ^5 -androstenediol (adiol) and Δ^5 -androstenediol-3-sulfate (adiol-3S) in serum and urine. Adiol was recovered from the samples by extraction with dichloromethane whereas the sulfates were extracted in ethyl acetate after deproteinization. After solvolysis adiol-3S could be assayed as free adiol. Extracts were purified on HPLC and concentrations determined by GC-MS after derivatization. Analytical performance of the assay was tested and proven to be adequate for clinical purposes.

Chapter 9 demonstrates the practical use of the assay. Reference values for adiol and adiol-3S were established and found to be higher for men than for women. In premenopausal women, adiol serum concentrations correlated with DHEAS, C, and A and were as well ACTH as LH dependent. In postmenopausal women adiol and adiol-3S concentrations were lower than in premenopausal women. Adiol levels in men correlate with testosterone concentrations. These results indicate that adiol and adiol-3S are both adrenal and gonadal derived steroids but that in postmenopausal women adiol and adiol-3S are predominantly of adrenal origin. It was found however that in several endocrine situations adiol and adiol-3S behave rather unpredictably in comparison with routinely measured adrenal steroids.

Chapter 10 shows the effect of two aromatase inhibitors (fadrozole and 4-hydroxy-

androstenedione) on estrogen metabolism. Aromatase inhibitors specifically block the conversion of A to estrone (E_1) and testosterone (T) to estradiol (E_2). E_1 , E_2 , adiol and their sulfates were assayed during treatment with either aromatase inhibitor in serum of postmenopausal women with breast cancer. The determination of adiol and adiol-3S was undertaken, because it is well established that adiol has estrogenic properties and thus, like estrogens, could promote tumor growth.

Although peripheral aromatase inhibition is almost complete, we found that circulating levels of estrogens in postmenopausal women were only reduced by approximately 40%. Nevertheless this reduction is apparently sufficient to produce a clinical effect. In addition, if intracellular concentrations are reduced comparably, estrogen dependent growth of the tumor is unlikely. Although adiol and adiol-3S levels in patients treated with each one of the two inhibitors did not change significantly, in individual patients large differences and sometimes elevations during therapy were observed. The occasional elevation of these steroids usually goes unnoticed, since adiol and adiol-3S are not determined on a routine basis. Higher concentrations of these estrogenic steroids could explain the poor response sometimes observed in patients despite substantially reduced estrogen levels.

Chapter 11 describes the optimization of the tritium release aromatase assay and its use in assessing aromatase activity in benign or malignant breast tissue. The assay, originally developed for assaying aromatase in placental tissue containing high activities of the enzyme, was initially not sensitive enough to estimate the low activities in breast tissue samples. In optimizing the assay, inhibiting factors responsible for the non linear tritium release curve were studied. By means of tritium release experiments and GC-MS, hydroxylated estrogens were found to be synthesized during the incubation. These steroids were found to inhibit the aromatase reaction. The addition of albumin, dithiothreitol and extra NADPH improved linearity and a more extensive procedure to separate tritium labeled water from the incubation mixture improved sensitivity. The improved assay was used to determine aromatase activity in several breast tissue samples. Aromatase activity was found in more than 50% of all tested samples. There were no quantitative differences in aromatase activities between adenoma, carcinoma and normal breast tissue samples. The normal group however contained significantly more aromatase positive samples than the adenoma and carcinoma group. Although aromatase positive carcinomas are expected to respond to aromatase inhibition

therapy, treatment can be started without knowledge of the aromatase status since a relatively high percentage of the breast carcinomas possesses aromatase activity. The described method could be used in future research programs to study the intracellular effect of aromatase inhibitors on local estrogen synthesis.